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PHYSIOLOGICAL INVESTIGATIONS OF RED RASPBERRY PLANTS INOCULATED WITH RED RASPBERRY MOSAIC

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Physiological Investigations of Red Raspberry Plants Inoculated with Red Raspberry Mosaic

BUFORD H. GRIGSBY*

INTRODUCTION

Virus diseases of the raspberry were first recognized in 1895 by Green (17) although it is said that Detmers (12) in 1891 described a disease that was very likely mosaic but was then ascribed to bacteria. Mosaics are responsible for the so-called "running out" of several varieties, many of which have been abandoned. Furthermore such troubles not only cause serious reduction in yields and quality of fruit but account for considerable decline in acreage. In 1922 Rankin and Hockey (31) divided the virus diseases into two groups, "curl" and "mosaic". Bennett (3) in a more recent study has reported at least five distinct virus diseases of the raspberry. They are, red raspberry mosaic, mild mosaic, yellow mosaic, curl and streak. The former is the one dealt with in these studies.

Bennett (3) also states that temperature has a very decided influence in masking symptoms of red raspberry mosaic and mild mosaic. At temperatures higher than 28° C., no symptoms of mottling are shown. Plants with symptoms masked, were therefore easily obtained when desired.

Numerous publications, summarized by Smith (36), dealing with symptoms, methods of dissemination and transmission, and other pathological phases have appeared, but the writer has found very little information on the effect of red raspberry mosaic virus on physiological activities of the host. It appeared worth while then to make comparative studies of the physiological behavior of healthy and mosaic-infected plants and also of plants in which the symptoms of red raspberry mosaic had been masked.

MATERIALS AND METHODS

In all of these studies the Latham variety was used. Healthy plants of this variety were inoculated with the virus responsible for the disease described by Bennett (3) as red raspberry mosaic and by Cooley (8) as green mottle mosaic. The symptoms produced vary considerably with the variety and with the environmental conditions. The leaves present a typical mosaic mottling characterized by yellowish green areas alternating with raised dark green areas, when the plants are

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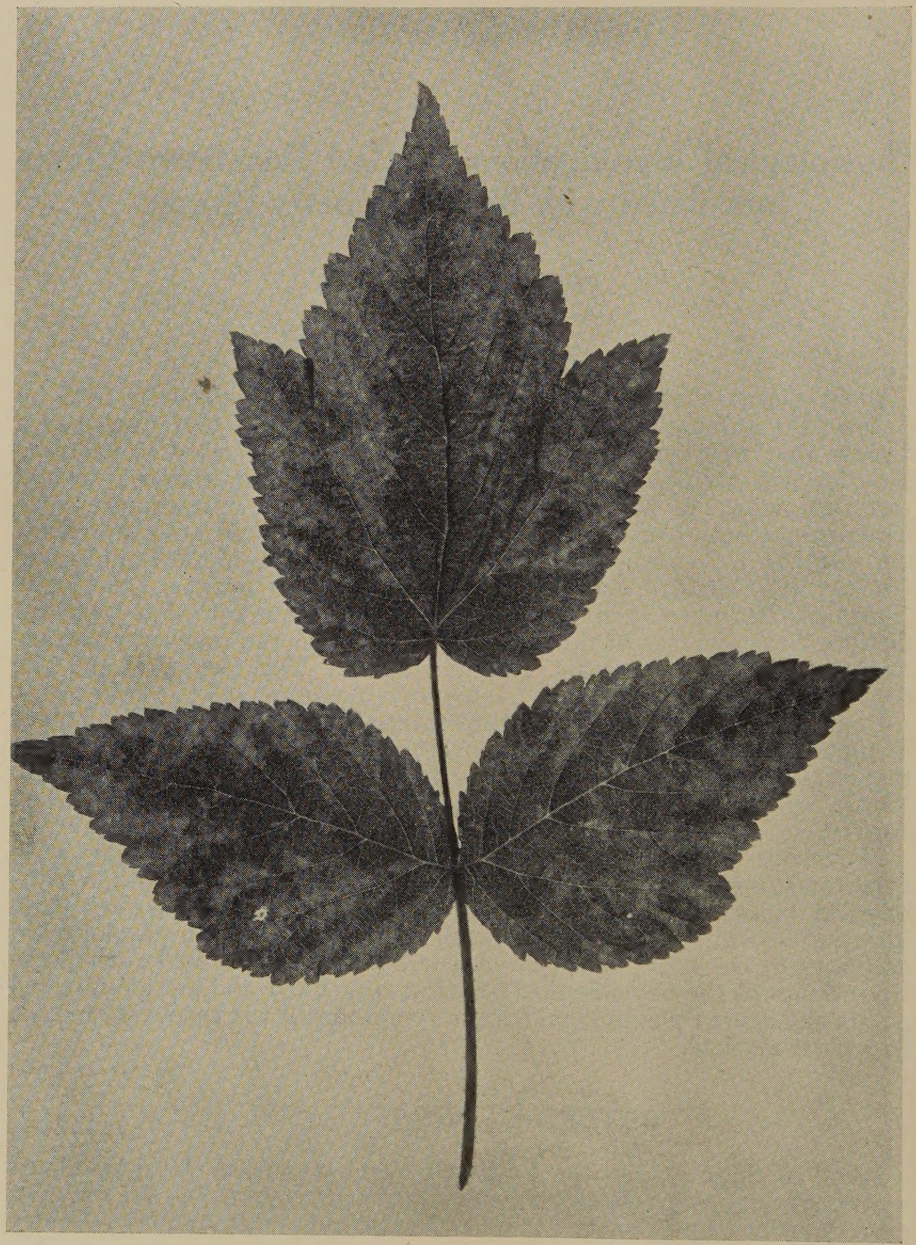

Fig. 1. Typical leaf of the Latham variety infected with red raspberry mosaic.

grown at a relatively cool temperature. Typical leaves of this sort are shown in Figs. 1 and 2. Under ordinary summer temperature in Michigan the mosaic-infected plants show no symptoms and the disease is said to be masked.

In the fall of 1932, 25 Latham plants inoculated with red raspberry mosaic were received from W. H. Rankin of the New York Agricultural Experiment Station at Geneva. These plants were grown in the greenhouse during the winter and transferred to the field in the following spring. While in the field they were covered with white muslin cages to prevent any possible infection from other sources. Additional diseased plants were obtained through the use of bark grafting, a

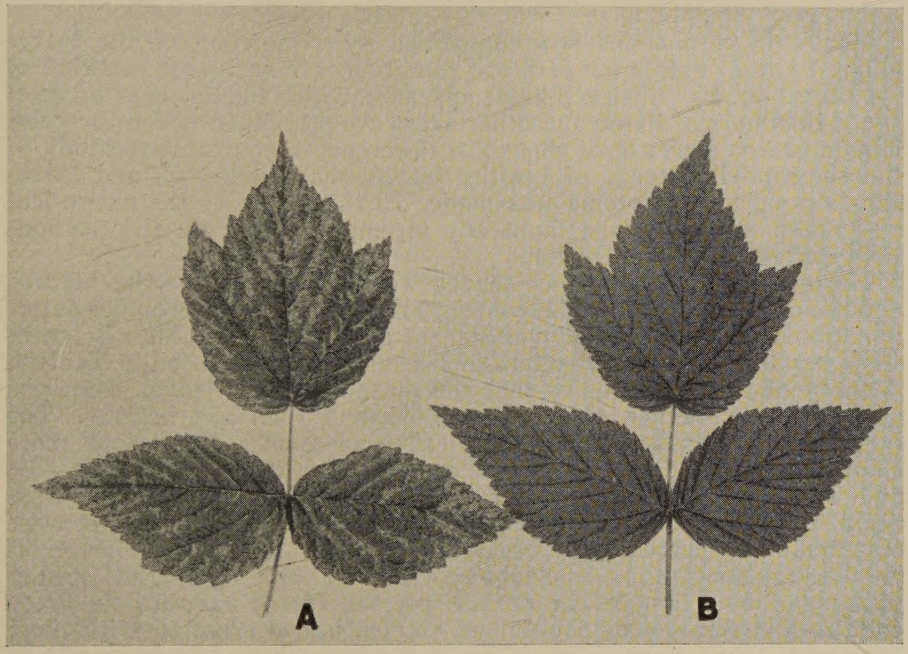

Fig. 2. Healthy and mosaic-infected leaves of the Latham variety. (A. Mosaic-infected) (B. Healthy)

method suggested to the writer by Harris of the East Malling Research Station, England. Since the results with this method have yielded more than 90 per cent infection the writer early gave up attempts to transmit the disease by insect vectors. During this time many methods were tried, as insect vectors were difficult to find during the earlier periods of this study. That difficulty was due to the hot, dry weather and other environmental conditions. Various inoculation methods for virus transmission were given a trial, especially the carborundum method. Every attempt in this direction failed. It has been suggested that the virus is destroyed by oxidation when juice from infected plants is used for inoculation.

The symptoms of the disease do not appear in the grafted plants

until a new set of shoots is sent up from the roots. In a comparatively short time these will appear when the plant is cut back. Harris, in a letter to Hibbard, states that in England symptoms do not appear on the grafted plants until the year after grafting, but that in St. Catherines, Ontario, where he (Harris) had made many grafts during 1934, red raspberry mosaic appeared within two months after grafting. No explanation was given to account for the difference obtained in the Canadian material. Plants for this study, in which the symptoms were masked, were obtained by selecting diseased plants and exposing them to proper temperature conditions.

CHLOROPHYLL

Mosaic in raspberries is accompanied by a mottling of the leaves (Figs. 1 and 2) unless temperature conditions are such that masking of symptoms occurs. Blistered areas appear in which the tissue is a darker green than normal tissue and other areas are present in which the color is pale green. Because of this color difference a comparative study of the chlorophyll content of healthy leaves, masked leaves, and leaves showing mosaic symptoms was made. In those studies the entire leaf was taken for analysis without any attempt to separate the portions with different shades of color.

The chlorophyll content of leaves was determined by the method described by Ulvin (42). The colorimetric method of chlorophyll determination was selected. The results are given in milligrams of chlorophyll per 100 square centimeters of leaf area and are noted in Table 1. A study of this table shows that the mosaic-infected leaves contained approximately 12 per cent more chlorophyll than the healthy. The leaves of the masked plants also possessed about 11 per cent more chlorophyll. These results do not agree with those of Peterson (30) and were unexpected and a little surprising. The experiments were again repeated in a different season and the same general results were obtained. The percentage increase is not great but is significant. A possible explanation is advanced as a result of some cytological studies of diseased leaf tissue. The mosaic-infected leaves have a pale green color and in some spots are almost yellow, but alternating with these areas are other numerous raised or blistered areas that are a very dark

TABLE 1. *Fresh Weight, Total Chlorophyll and Chlorophyll per 100 sq. cm. in Leaves of Healthy Masked and Mosaic-infected Plants.*

Plant	Fresh Weight Gm.	Area Cm.²	Weight 100 Cm.²	Chlorophyll Mg.	Chlorophyll 100 Cm.²	Average Chlorophyll 100 Cm.²	Ratio
Healthy.....	8.127	324	2.54	8.94	2.76	——	——
Healthy.....	5.132	322	1.60	7.96	2.47	2.61	100
Masked.....	4.152	204	2.07	6.30	3.15	——	——
Masked.....	4.044	196	2.02	5.69	2.79	2.97	111
Mosaic.....	3.301	144	2.20	4.78	3.18	——	——
Mosaic.....	3.358	162	2.09	4.78	2.98	3.08	112

green in color. A microscopical study of sections in these blistered areas showed two or three extra layers of palisade cells, all of which contained numerous plastids. These extra cells are apparently plentiful enough to give the diseased leaves more chlorophyll than healthy ones. Bennett (3) states that the severely affected varieties of red raspberries "show a fine-grained pattern of mottling consisting of small yellow spots surrounded by tissue of deeper green color. Leaves in general are deeper green than are leaves of healthy plants." The two tests are indicative of a small increase in chlorophyll in the mosaic-infected leaves.

Peterson (30) in 1931 found a reduction in chlorophyll content in mosaic-infected tobacco. Chloroplasts in leaves of mosaic-infected plants have been studied and the changes noted, but little or no knowledge is obtainable on the chlorophyll content and its possible variation in diseased and healthy plants. In connection with the diseased plant's reduced photosynthetic activity, one might expect a reduction in chlorophyll content. However, reduced photosynthetic activity might just as well be due to accumulation of products or some other condition.

CARBOHYDRATE STUDIES

A number of investigators have reported differences in carbohydrate and nitrogen relationships of plants infected with virus diseases. In this work carbohydrate analyses were made to determine the differences, if any, between healthy plants and those infected with the virus. Early tests were made on plants grown in the greenhouse, and later field-grown plants were used. Analyses were made on leaf and root tissues. In the leaf studies leaves from healthy plants, plants showing definite symptoms, and plants in which the virus was present but the symptoms masked were used. In the analyses of root tissue masked plants were not used.

Leaves of approximately the same age were obtained from three sorts of plants. The material was collected at 10 a. m., dried in a gas oven at 100° C. for one hour, then at 60° C. until constant weight was reached. The entire samples were then ground to pass through a 40-mesh screen and analyses made according to the routine suggested by the committee on chemical methods of the A.S.P.P. (33). Bertrand's (4) permanganate titration method was substituted in place of that of Shaffer and Hartman.

Roots from healthy and mosaic-infected plants were dug and carefully washed to remove adhering soil particles. The samples were then dried and analyzed in the same way as the leaves.

The results of these analyses are shown in Table 2. The data in this table show that field-grown plants infected with the virus usually have smaller percentages of carbohydrates than do greenhouse-grown plants. In the case of healthy plants, the reverse is true. The results of various investigators show little agreement regarding the influence of a virus upon the total carbohydrates of plants. Dunlap (15) reported an increase in total carbohydrates of two-year-old raspberry canes having mosaic but showed a decrease in one-year-old canes. In other mosaic-infected plants such as tobacco, squash, tomato, cucumber, he found a decrease in carbohydrates. Rosa (35) found an increase in

carbohydrates of the leaves of tomato plants infected with the yellow blight virus. True and Hawkins (41) found greater quantities of carbohydrates in blighted spinach leaves. Brewer, Kendrick, and Gardner (7) showed a decrease in carbohydrates in leaves of mosaic-infected tomato plants. In all these cases no attempt was made to determine the various carbohydrate fractions.

Examination of Table 2 shows that in leaves of field-grown plants the mosaic-infected plants have more sugars than either of the groups. Healthy leaves contained the least and masked plants were intermediate. Glucose is more abundant in greenhouse plants than in field-grown plants when the virus is present. Healthy plants in either case contain more glucose than either of the other groups. Sucrose is present in greater quantities in mosaic-infected and masked leaves than it is in healthy leaves. These results are in agreement with those of certain other investigators. For example, Barton-Wright and McBain (1) found more sucrose than glucose in the crinkle-infected potato. True and Hawkins (41) reported a reduction in amount of hexoses and an accumulation of sucrose in leaves of blighted spinach. Reduced amounts of hexoses were also reported by Dunlap (15) in the leaves of mosaic-infected tobacco. The differences in all cases are attributed to a slowing-up of the processes of translocation in the virus-infected plants.

Regarding starch, the table shows that healthy leaves have more starch than do masked and mosaic plants. The decrease is evident in the soluble amylodextrin fraction as well as in the insoluble or amylocellulosic form but greatest reduction occurs in the amylodextrin form. Only slight differences are shown between field-grown and greenhouse-grown leaves but the field-grown leaves usually have the lower values. Other investigators have reported similar results in other plants. Brewer, *et al.* (7) found a decrease in the amount of starch in mosaic-infected tomatoes. Cook (8) reported an inverse relationship between starch and the severity of sugar cane mosaic. Bolas and Bewley (7) observed a decrease in rate of starch formation in the tomato plant when the plant had been infected for several weeks. In early stages of the disease there was no appreciable change in starch formation as compared to that of healthy plants.

Study of that part of Table 2 dealing with the carbohydrates of roots shows that here the carbohydrate picture is markedly different from that of the leaves. With the exception of simple sugars, all carbohydrates are present in much greater quantities in roots than in leaves. The roots of field-grown plants were taken at the end of a growing season and those of greenhouse plants at a time when growth and development of new shoots was just getting under way, therefore, one should expect to find greater quantities of carbohydrate material in the roots of the plants at the end of the growth period.

Greater quantities of all carbohydrates are shown to be present in healthy roots than in roots of diseased plants. Glucose appears to be not unlike the amount found in leaves, but all other fractions in both healthy and diseased roots are much more abundant in the roots. Sucrose in the roots is present in greater quantities in healthy plants but in leaves the higher values for this fraction occur in plants containing the virus.

TABLE 2. *Percentage of Carbohydrates in Leaves and Roots of Healthy Plants, Mosaic-infected Plants and Plants in which Symptoms were Masked.*

LEAVES

Date	Hour	Plant and Habitat	Sugar		Total Sugar	Starch		
			Simple	Sucrose		Soluble	Insoluble	Total
July 20...	10 A.M.	Healthy, greenhouse.	0.64	0.74	1.38	1.00	.73	1.73
July 20...	10 A.M.	Healthy, greenhouse.	.64	.73	1.37	.95	.71	1.66
Aug. 21...	10 A.M.	Healthy, greenhouse.	.69	1.19	1.88	.52	.97	1.49
Aug. 21...	10 A.M.	Healthy, greenhouse.	.64	1.18	1.82	.50	.92	1.42
Aug. 21...	10 A.M.	Healthy, field.....	.70	1.22	1.92	1.07	.68	1.75
Aug. 21...	10 A.M.	Healthy, field.....	.67	1.22	1.89	1.07	.70	1.77
Aug. 26...	10 A.M.	Healthy, field.....	.65	1.39	2.04	.37	.56	.93
Aug. 26...	10 A.M.	Healthy, field.....	.64	1.43	2.07	.38	.55	.93
July 20...	10 A.M.	Mosaic, greenhouse.	.59	1.20	1.79	.50	.85	1.35
July 20...	10 A.M.	Mosaic, greenhouse.	.60	1.18	1.78	.50	.85	1.35
Aug. 21...	10 A.M.	Mosaic, field.....	.57	1.72	2.29	.47	.87	1.34
Aug. 21...	10 A.M.	Mosaic, field.....	.57	1.75	2.32	.50	.86	1.36
Aug. 21...	10 A.M.	Masked, greenhouse.	.75	1.29	2.04	.65	.80	1.44
Aug. 21...	10 A.M.	Masked, greenhouse.	.75	1.29	2.04	.65	.80	1.45
Aug. 26...	10 A.M.	Masked, field.....	.57	1.45	2.02	.47	.59	1.06
Aug. 26...	10 A.M.	Masked, field.....	.57	1.45	2.02	.47	.67	1.14

ROOTS

Oct. 22...	10 A.M.	Healthy, field.....	0.75	2.65	3.35	1.85	4.85	6.70
Oct. 22...	10 A.M.	Mosaic, field.....	.60	2.50	3.10	1.55	4.35	5.90
Feb. 3...	10 A.M.	Healthy, greenhouse.	.60	2.50	3.10	1.60	2.10	3.70
Feb. 3...	10 A.M.	Mosaic, greenhouse.	.20	2.30	2.50	2.00	2.70	4.70

In the case of the greenhouse plants, both glucose and sucrose are found in decreased amounts in the diseased roots as compared to healthy plants. Soluble and insoluble starches, however, are more abundant in the diseased roots. The presence of the virus evidently exerts considerable influence upon the conversion of reserve carbohydrates into soluble forms.

There are no data available in regard to the carbohydrates in roots of raspberry with virus diseases but reports have been made of analyses of the stems of many plants. Dunlap (15) found an increase in carbohydrates of old raspberry canes infected with mosaic. Rao and Sreenivasaya (32) reported an increase of starch in the stem of spiked sandal. While stems and roots are not comparable structures it appears to the writer that an analogy can be drawn between them in this case. Both are storage regions and the results reported here are certainly closely related to storage and utilization of storage forms of carbohydrates.

These data then may be considered as being in agreement with the results of Rao and Sreenivasaya (32) and in partial agreement with the results of Dunlap (15). Dunlap's analyses were made on growing shoots and thus the plants may have been in condition similar to that of the greenhouse plants used in this work.

The difference in carbohydrate metabolism in the mosaic-infected plants as compared with that in healthy plants has been attributed by most workers to the interference in transport rather than to inability to produce carbohydrates. In plants where one effect of the virus is to cause a yellowing of the photosynthetic tissue it is quite probable that carbon assimilation is reduced, but for many of the mosaic diseases

this explanation is not valid, for in many plants there is very little yellow-colored tissue, while in some cases the green color is more intense. It was thought that a study of the carbohydrate changes in a raspberry plant during a 24-hour period might give information as to whether production or transport was being checked.

For this experiment, field-grown plants were used and samples of leaves were collected early in the morning before sunrise and again the same day just before dark. The samples were selected in such a way that material from the same plants was being analyzed in each case. The fourth and fifth leaves back from the tip are usually fully expanded and in this case were considered mature. By taking, then, the fourth leaf of one cane and the fifth leaf of another cane in the morning it is possible to obtain a sample in the evening from the same canes which is comparable in every respect to those taken previously. The samples were analyzed by the same method indicated previously, and the results are given in Table 3.

TABLE 3. *Changes Occurring During Day and Night Periods in the Percentage of Carbohydrates in the Leaves of Healthy, Mosaic-infected Plants and Plants in which the Symptoms are Masked.*

Plant	Simple Sugar		Ratio		Sucrose		Ratio		Soluble Starch		Ratio		Insoluble Starch		Ratio	
	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise	Sunset	Sunrise
Healthy.....	2.00	1.60	100	100	3.52	4.18	100	100	2.09	1.77	100	100	0.88	0.69	100	100
Healthy.....	2.10	1.59	....	....	3.53	4.18	....	....	1.99	1.75	....	....	.90	.67	....	....
Mosaic.....	1.49	1.10	72	69	3.58	4.35	101	103	2.15	1.72	105	97	.89	.89	100	130
Mosaic.....	1.49	1.13	72	70	3.60	4.37	102	103	2.12	1.72	104	97	.93	.95	104	139
Masked.....	1.79	1.72	87	107	3.52	3.70	100	89	1.81	1.73	88	97	1.45	1.26	163	182
Masked.....	1.79	1.75	87	107	3.52	3.63	100	88	1.80	1.80	88	102	1.40	1.26	157	182

The values shown in the column headed "SUNRISE" are for the samples obtained before sunrise and represent the carbohydrate fractions in the leaves after a period of 11 hours during which no photosynthesis had taken place. Obviously these changes are chiefly related to translocation and utilization. The figures indicated in the column headed "SUNSET" are for the samples gathered in the evening and represent the carbohydrate fractions in the leaves as the result, chiefly, of photosynthesis.

A study of the table shows that healthy plants produce more simple sugars during the daytime than either the masked or mosaic-infected plants. This indicates a greater efficiency in sugar production. During the night there is a greater loss of these sugars in healthy plants than in the diseased ones, indicating efficient transport and more rapid transformation. When the sucrose figures are considered all groups of plants show similar amounts, with slightly more occurring in the mosaic-infected plants in the daylight period. From this it may be concluded that sucrose is maintained at a fairly constant level. During

the night, however, the sucrose content increases in all groups, with the greatest increases occurring in the diseased plants.

Soluble starch is more abundant during the daytime period in the mosaic-infected plants. During the night it is somewhat lower. In all three groups the soluble starch has decreased during the night showing that it has been utilized in some biochemical reaction. The insoluble starch fraction in the mosaic-infected plants is the same for day and night periods. The healthy and masked plants show significant differences between day and night periods.

To summarize, it is readily seen that sucrose remains relatively constant during the day, and that also, as is shown in Table 2, the diseased plants contain more of this sugar than the healthy ones.

The increase in sucrose during the night is in part at the expense of the simple sugars. The sunrise values for simple sugar are considerably lower than the sunset values while the sunrise values of sucrose are higher than the sunset values. For the mosaic-infected plants this accounts for approximately half of the increase in sucrose. The other half is accounted for by the transformation of the soluble starch fraction (dextrin) into sucrose for, as may be seen in the table, the sunrise values of soluble starch are compensatingly less than the sunset values. A similar line of argument is applicable in the case of the healthy and masked plants. The data in the column headed "Insoluble Starch" are difficult to interpret. Had the values been higher in the nighttime for healthy plants and had showed no change or lower for the masked and mosaic-infected plants, it would seem to prove that the diseased plants were inefficient in transforming the sugars and soluble starches into permanent structures.

Support for the idea that sucrose is increased at the expense of glucose and starch, is found in the studies of Barton-Wright and McBain (1) who explained the increase of sucrose in potatoes infected with crinkle mosaic to a loss of hexoses and starches. They showed also that the sucrose was produced from glucose and fructose (hexoses). The results reported in Tables 2 and 3 do not support the idea of checked or retarded translocation since a considerable increase in sucrose occurred in the healthy plants as well. Cook (8) also found no interference in translocation in the mosaic-infected sugar cane.

NITROGEN DETERMINATIONS

Part of the leaf samples collected for carbohydrate determinations were used for nitrogen analyses. Later roots were collected and nitrogen determinations made from this material also.

Analyses were made for nitrogen in the form of ammonia, nitrate nitrogen and total nitrogen only. The usual Kjeldahl procedure was used as outlined by Loomis and Shull (27). Results of these analyses are shown in Table 4.

The data in this table show that leaves of healthy plants have a higher percentage of total nitrogen than either masked leaves or leaves in which the symptoms are evident. Rosa (35) reported similar results in tomato leaves infected with yellow blight. He also found higher total nitrogen for other parts of the tomato plant. Jodidi, *et al.* (22) (23) found low total nitrogen in the tops of mosaic-infected spinach

TABLE 4. *The Percentage of Ammonia, Nitrate Nitrogen and Total Nitrogen in Leaves and Roots of Healthy Masked and Virus-infected Raspberry Plants.*

	NH_3	NO_3	Total		NH_3	NO_3	Total
Healthy, Leaf				Mosaic, Leaf			
July 18	1.20	2.00	3.20	July 18	2.30	.30	2.60
July 31	2.10	.80	2.95	July 31	2.45	.60	3.05
Aug. 26	1.70	.90	2.60				
Average	1.66	1.23	2.92	Average	2.37	.45	2.84
Masked, Leaf				Healthy Roots			
July 31	2.40	.30	2.70	Oct. 22	.70	.30	1.00
Aug 26	2.20	.20	2.40				
Average	2.30	.25	2.35	Mosaic Roots			
				Oct. 22	.80	.30	1.10

and cabbage. Rao and Sreenivasaya, however, reported a higher nitrogen content in mosaic areas of sandal stems. Dunlap (15) found an increase in total nitrogen in young raspberry canes infected with mosaic.

Turning to a consideration of the various nitrogen fractions, research shows that leaves of all plants containing the virus, with symptoms evident or masked, have a greater percentage of ammonia than do healthy leaves. At the same time these virus-infected plants show a decrease in percentage of nitrate nitrogen. The greater quantity of ammonia is probably due to two factors. In the first place, the decrease of nitrates shows that this form of nitrogen is affected by the virus. Breaking-down of nitrates can lead to the formation of ammonia in plants. Another factor is the failure of the plant to utilize ammonia in protein synthesis, and as a result ammonia accumulates in the plant. These results agree with those of Jodidi, *et al.* (22) (23) who studied the nitrogen metabolism of cabbage and spinach plants infected with the mosaic virus.

Those plants in which the symptoms were masked are of interest because of the similarity in nitrogen content to that of the plants with symptoms evident. In the carbohydrate studies reported in this paper it has been shown that the carbohydrate metabolism of masked plants is very much the same as in healthy plants. Thus the presence of the virus apparently exerts a more marked influence upon nitrogen metabolism than it does upon carbon metabolism.

Results of the root analyses are of little value in this case except that they show that roots have lower percentages of nitrogen than leaf tissue. Differences between healthy and diseased roots are too slight to be significant.

ENZYME STUDIES

The differences in carbohydrate metabolism between the healthy and diseased plants suggested a possible connection with enzyme activity or inactivity. Only qualitative or approximate quantitative tests were possible in the two experiments conducted at two different seasons. Enzyme extracts were obtained from leaves of similar age, selected from the three different groups of plants. The leaves were gathered in

the late morning period. The enzymes were extracted by a method described by True and Hawkins (41) in which glycerine was the extracting medium. A preliminary grinding of the tissues with clean quartz sand was necessary to rupture the cell walls. The glycerine extraction was continued for 24 hours at a temperature of 10° C. The extract was then squeezed through cheesecloth and made up to mark in a 250 cc. volumetric flask. A one-per-cent Lintner's starch solution was made up fresh each time a test was conducted. A test consisted of placing varying concentrations of the enzyme solution in a series of test tubes and then adding 5 cc. of a one-per-cent starch solution and one-half cc. of toluol to each tube. The tubes were closed with a stopper, shaken and then placed in an incubator at 37° C., for 48 hours. At the end of this time the tubes were removed from the incubator, immersed in cold water and to each were added 10 cc. of cold distilled water and one-tenth cc. of a one-per-cent iodine solution. The tubes were then shaken and the enzymatic activity determined by the observation of changes in color. The data may be found in Table 5.

TABLE 5. *Diastatic Activity of Extracts from Leaves of Healthy, Masked, and Mosaic-infected Plants, as Determined by the Color Produced by the Addition of Iodine to a Starch Substrate. (Zero Indicates Greatest Activity, and 100 no Activity.)*

Plant	Quantity of exzyme extract					Control
	0.5 cc.	0.4 cc.	0.3 cc.	0.2 cc.	0.1 cc.	
Healthy	20	40	60	80	100	100
Healthy	20	40	60	80	100	100
Masked	20	40	60	80	100	100
Masked	20	40	60	80	100	100
Mosaic	0	20	50	60	100	100
Mosaic	0	20	50	60	100	100

The tube containing the lowest dilution of enzyme, in which no blue color was found on treatment with iodine, was arbitrarily taken as zero. Then the tube into which a small quantity of boiled enzyme had been introduced, and which on treatment with iodine, showed the blue color, was taken to represent the other end of the scale or 100. The other tubes were graded according to color on a percentage basis. From the table it is obvious that the diastatic activity of the leaves of mosaic-infected plants was greater than that of the masked or healthy ones. When considered with the carbohydrate changes already described, the increased diastatic activity explains why there was such a small amount of soluble starch or dextrin and why there was an increased amount of sucrose in the mosaic-infected plants. In the case of the masked plants it appears that diastatic activity has not been modified. Masking effects in the raspberry may be in some phases comparable with the effects of a latent virus in the metabolism of a potato, for example. Barton-Wright (2) reported that potatoes containing a latent virus had in no way modified carbohydrate metabolism. In that case no changes in enzyme action would be expected.

Proof that diastatic activity is increased in mosaic-infected tobacco has been shown by Dunlap (15). Rao and Sreenivasaya (37) found that the diastatic activity of spiked leaves of sandal was much greater than that of the healthy leaves. True and Hawkins (41) however were unable to detect any difference between the diastatic activity of the leaves of healthy and diseased spinach. Raspberries then appear to be not unlike other plants infected with mosaic in their power to quickly convert starch and also glucose into sucrose.

PHOTOSYNTHESIS AND RESPIRATION STUDIES

The rate of photosynthesis in leaves, or their efficiency in the food manufacturing process, has been determined for many types of plants under varying climatic conditions, and experimental procedures. It has been shown that not only do external conditions modify this process, but internal conditions also, such as water and chlorophyll content, age, variations in the ability to react (protoplasmic fatigue), improper nutrient conditions, translocation and utilization of assimilated materials. Despite those modifying conditions, it is not impossible to obtain a fair approximation of a normal leaf's efficiency. The object of this work was to compare the photosynthetic efficiency of healthy raspberry leaves and those infected with mosaic. The method of Heinicke and Hoffman (19) as applied to apple leaves was selected as a guide for these studies. In principle it is like many others dating back even to the time of Kreusler's work in 1885, but it has the advantage of being useful in cases where the leaves are still attached to the plant. These authors have also been very explicit in their descriptions of the method, and in pointing out the various steps and the need for care and practice in developing technique. This is important as it is then possible to duplicate procedures, a state of affairs which has usually been impossible to accomplish in experiments of this type.

The report that follows deals with plants grown in the greenhouse. The foregoing carbohydrate studies reported have shown that normal healthy plants exhibit the same general type of metabolism in the greenhouse as in the field. The field plants, however, produce approximately twice as much material. Similar studies by other workers have also been conducted in greenhouses and no marked variation in the type of carbohydrate metabolism has been thus far reported. First studies are naturally made where experimental facilities are available and where complicated apparatus can be set up without fear of its being disturbed. Three series of experiments were carried through, one in the spring of 1936, a second in the summer of the same year and the last in the spring of 1937.

Photosynthetic efficiency was estimated by determining the difference in CO_2 content between a continuous stream of normal air and a similar stream of air that had passed over leaf tissue confined in a specially constructed photosynthetic chamber. The photosynthetic chambers were in the form of cylinders made of celluloid. As shown in Fig. 3 they were large enough to inclose the entire end of a raspberry cane. Thus a considerable amount of photosynthetic tissue can be used and crowding of leaves avoided. There was also a minimum of tem-

perature variation so prevalent in experiments where single leaves or portions of leaves are used in cup chambers or other small containers.

The cylindrical chambers were 20 inches high and 30 inches in circumference. A celluloid cover was cemented to one end with a solution of celluloid in acetone. All joints at this end were sealed tight. At the other end the cylinder was attached to a metal rim to which was fastened a long metal rod that could be used for a support when stuck in the ground. Cellophane fastened to this rim by an elastic band, and loosely tied around the exit and inlet air tubes and stem, closed up the chamber. The absorption units with slight modification were the same as those pictured and described by Heinicke and Hoffman (19) on page

Fig. 3. Absorptions unit and assimilation chambers as set up for operation. (A. Celluloid assimilation chamber.)

four of their publication. Their general method of procedure as to calibrations, taking of samples, regulating air supply, titrations, was followed. The running time of an experiment was always for four hours as this was a convenient time interval for the operator and was of sufficient length to produce recognizable quantities of CO_2. Similar runs for the same time periods were made in darkened chambers or during the night in regular chambers for information on respiratory activity in leaves.

Leaf areas had to be determined and this was accomplished without injury to the leaf or plant by means of a simplified and portable photo-electric device described by Hibbard, Grigsby, and Keck (21). This device has been used for three years and has a few special features not

yet noted in the descriptions of several devices recently published. It is operated on a 6-volt battery, has a multiple point, low intensity light source, and a specially constructed cone-shaped light chamber. It needs no correction for transmission of light through the test leaf. The device can be made portable, and an automobile battery can be used as the source of power (Fig. 4).

The data for photosynthesis and respiration may be found in Tables 6, 7, and 8. The amounts of CO_2 shown in these tables represent, for the day period, the reduction in quantity of CO_2 in the stream of air drawn over the leaves as compared with the amount of this gas in the air drawn through the control chamber which contained no leaves.

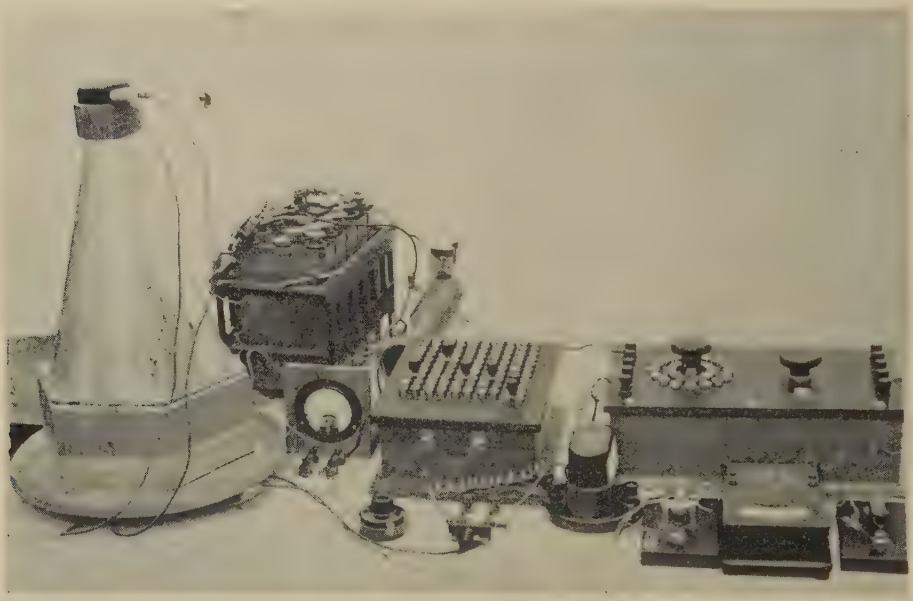

Fig. 4. Photograph of leaf area apparatus as set up for operation.

For the night period the values represent the increase in CO_2 as compared with that in the control chamber. This increase is due to the CO_2 given off in the processes of respiration while the decrease during the day periods represents the difference between the amount of CO_2 absorbed for the purpose of photosynthesis and that set free by respiratory activity during the same period. This is, therefore, not a true measure of the total photosynthesis but is called here, "apparent photosynthesis," and can be used for the purpose of comparison between healthy and diseased leaves. This is the general interpretation of the above expression. In the tables "a.m." and "p.m." represent day readings, forenoon and afternoon respectively, while "NIGHT" refers to readings made after it became dark in the evening.

Greenhouse temperatures between night and day are usually of wide variation but a system of regulation was possible and in these experiments the greatest variation in temperatures was not more than 5° F.

TABLE 6. *Apparent Photosynthesis and Respiration of Healthy and Mosaic-infected Plants in the Spring of 1936, Expressed as Milligrams of CO_2 per 100 sq. cm. of Leaf per Hour. Average of 4-hour Determinations During Day or Night.*

Date	Plant	Day	Night	Ratio	
				Day	Night
May 4	Healthy	6.50	4.78	100	100
4	Mosaic	4.15	5.28	63	110
11	Healthy	17.79	5.23	100	100
11	Mosaic	9.97	6.07	77	115
13	Healthy	15.31	4.62	100	100
13	Mosaic	9.09	6.10	59	132
15	Healthy	6.52	2.78	100	100
15	Mosaic	3.82	3.56	58	128
Average	Healthy	11.53	4.35	100	100
Average	Mosaic	6.76	5.25	64	121

TABLE 7. *Apparent Photosynthesis and Respiration of Healthy, Mosaic-infected and Masked Plants, Expressed as Milligrams of CO_2, per 100 sq. cm. of Leaf Surface per Hour. Average of 4-hour Determinations During Day or Night. Summer of 1936.*

Date	Plant	Day		Day Average	Night	Ratio	
		A.M.	P.M.			Day	Night
July 8	Healthy	11.70	10.75	11.22	4.06	100	100
8	Masked	13.09	11.79	12.44	4.61	110	113
8	Mosaic	10.50	9.69	10.09	5.05	89	121
9	Healthy	8.08	8.08	8.08	4.41	100	100
9	Masked	8.45	7.71	8.08	4.07	100	92
9	Mosaic	7.70	6.81	7.25	4.47	88	101
10	Healthy	12.12	13.22	12.67	4.07	100	100
10	Masked	12.13	12.13	12.13	4.03	95	99
10	Mosaic	11.62	12.07	11.84	4.53	93	111
14	Healthy	31.64	24.90	28.27	4.60	100	100
14	Masked	29.10	22.44	25.77	5.46	91	118
14	Mosaic	27.49	19.49	23.49	6.06	83	131
15	Healthy	17.22	15.68	16.45	5.54	100	100
15	Masked	17.94	17.37	17.60	7.46	107	134
15	Mosaic	13.24	12.10	12.67	8.97	77	161
16	Healthy	16.71	18.02	17.36	7.19	100	100
16	Masked	20.02	19.86	19.94	8.54	114	110
16	Mosaic	16.51	17.14	16.82	12.07	96	167
17	Healthy	16.90	15.94	16.42	6.41	100	100
17	Masked	20.07	17.40	18.73	7.55	114	117
17	Mosaic	15.00	14.82	14.91	8.90	84	138
Aug. 18	Healthy	17.32	15.50	16.41	5.48	100	100
18	Masked	17.83	17.17	17.50	8.02	106	146
18	Mosaic	15.96	15.00	15.48	9.24	94	168
23	Healthy	13.49	12.37	12.93	4.64	100	100
23	Masked	12.16	12.16	12.16	4.64	94	100
23	Mosaic	11.38	10.73	11.05	5.00	85	107
Average	Healthy	16.13	14.94	15.53	5.15	100	100
Average	Masked	16.75	15.33	16.04	6.04	103	114
Average	Mosaic	14.38	13.09	13.74	7.13	87	134

TABLE 8. *Apparent Photosynthesis and Respiration of Healthy and Mosaic-infected Plants in Early Spring, 1937. Results Expressed as Milligrams of CO_2 per 100 sq. cm. of Leaf per Hour. Average of 4-hour Determinations During Day or Night.*

Date	Plant	Day		Day Average	Night	Ratio	
		A.M.	P.M.			Day	Night
Mar. 29	Healthy	5.54	8.92	7.23	3.00	100	100
29	Mosaic	2.43	5.21	3.82	4.43	52	144
30	Healthy	4.17	—	4.17	1.32	100	100
30	Mosaic	1.38	—	1.38	2.73	33	206
Apr. 1	Healthy	7.91	5.54	6.72	3.41	100	100
1	Mosaic	4.19	2.86	3.52	3.58	52	105
2	Healthy	5.50	5.50	5.50	1.17	100	100
2	Mosaic	4.23	4.49	4.36	1.35	79	115
Average	Healthy	5.78	6.65	6.21	2.22	100	100
Average	Mosaic	3.06	4.18	3.42	3.02	54	142

The canes used were in their first season of growth. A study of all three tables shows that the apparent photosynthesis is reduced in the mosaic plants. In other words, the diseased plants are less efficient in photosynthesis than the healthy ones and also their rate of activity is reduced. Respiration on the other hand proceeded most rapidly in the mosaic-infected plants. The respiratory activity of the masked plants is intermediate, less than the diseased but more than the healthy.

A further study of the tables shows that photosynthetic activity varies with the time of the year. The spring or May values are lower than are the summer values. At least two explanations are possible. First, it might be a case of difference in vigor, the summer plants being the more vigorous or, secondly, it might be a group of external conditions, chiefly light and temperature that cause the difference. The former explanation does not appear to be as reasonable as the latter, since the type of growth in the raspberry is indeterminate, and the groups of leaves on the ends of the canes are in similar growth stages. Seasonal variations in photosynthetic rate do occur according to Dastur and Desai (10). Heinicke and Hoffman (19) reported variations from day to day. Bolas and Melville (6) observed a seasonal variation, the rate of photosynthesis being high in summer, and then declining towards winter. Variation is the rule rather than the exception.

Stone (39) working with the mosaic-infected potatoes found a reduction in photosynthetic efficiency, but noted that the severity of the disease had little effect upon the degree of reduction. Dufrenoy (14) claimed that mosaic decreased photosynthetic efficiency. Barton-Wright (2) found that in young potato plants suffering from mosaic there was no difference in carbohydrate metabolism or the ability to make carbohydrates, but as the plants got older there was a decline in the amount of carbohydrates formed. There seems to be no doubt in the case of the raspberry of a decrease in activity. The degree of reduction or decrease of activity seems to be proportional to symptom expression. In the masked plants the reduction is not so great as in the mosaic-infected ones. Whitehead (43) obtained similar results in potato plants infected with leaf roll. Dunlap (15) gives data that show an increased

rate of respiration in young raspberry plants and a decrease in older ones. His results are based on CO_2 evolved per hour in an average-sized leaf, the area of which was unknown.

TRANSPIRATION

A comparative study of the transpiration rate of healthy, diseased and "masked" plants was undertaken in the greenhouse. All records here, were obtained by the standardized hygrometric paper method. This as is known was first suggested by Stahl (38) and later was standardized by Livingston and his co-workers (25). There have been some modifications by Livingston himself and by other workers in this field in more recent times. The modifications by Meyer (28) were followed in the main. By using this method the leaves did not have to be detached from the plant and one can make tests on leaves under different conditions while they are growing naturally in benches or pots in the greenhouse. The method of obtaining results is more simple, and quicker than experiments with potometers or water culture set-up. It is preferable, especially in experiments of a preliminary nature, to the more elaborate methods which have been described.

Results shown in Table 8 are recorded as grams of water vapor lost in one hour per 100 square centimeters of leaf area. The relation between the color of the hygrometric paper and the amount of water taken up is determined in the following way. The time, in seconds, necessary for a definite area of paper pressed on the leaf surface, to attain the same pink color as a similar piece of paper in a saturated atmosphere and used as a reference standard, was found. Now, by using the formula $G = \frac{(0.06784 \times 3600)}{T}$ the amount of water vapor can be determined. The calibration of the reference standard showed that the pink color was equivalent to the absorption of 0.0678 grams of water vapor per 100 square centimeters of paper surface. Thus the color was a measure of water content of the paper and this was related to the time. In the equation, G equals the grams of water vapor

TABLE 9. *Grams of Water Vapor Transpired from Leaves of Healthy, Diseased, and Masked Plants per Hour per 100 Square Centimeters of Leaf Area.*

Time	Healthy (H)		Masked (M)		Diseased (D)		Ratio $\frac{(D)}{(H)}$		Ratio $\frac{(M)}{(H)}$	
	Bench	Pot	Bench	Pot	Bench	Pot	Bench	Pot	Bench	Pot
Mar. 13	1.95	—	1.95	1.08	2.03	1.14	104	—	100	55
20	1.37	—	2.01	1.08	2.00	0.76	145	—	145	78
21	—	—	—	1.30	1.78	1.11	166	—	—	122
31	1.01	—	—	.83	1.77	.95	176	—	—	82
Apr. 8	1.55	—	1.87	1.67	1.57	.95	101	—	120	107
12	—	—	—	—	1.63	1.25	140	—	—	—
Average	1.47	—	1.94	1.19	1.80	1.03	138	—	121	88

lost per hour, and T is the time of color change in seconds. The number of seconds in one hour is 3600, the figure indicated in the numerator in the equation above.

The figures in the table show that the mosaic-infected plants have a greater transpiration rate than the healthy ones and that the results from the masked plants are intermediate. Heuberger and Norton (20) observed a greater rate of transpiration in mosaic-infected tomato plants. Harrison (18) reported no increased transpiration in mosaic-infected bean leaves, but water losses from the pods of diseased plants were greater than from those of healthy plants.

The data also show that the loss of water vapor is more rapid in plants growing in the soil in an open bench where the soil is two feet deep, than in pots where the soil mass is restricted and not more than four or five inches deep. In the latter the supply of moisture is less than in the bench and the plant roots quickly use up the supply. When the plants have grown in pots for some time the majority of the roots grow near the sides of the pot and on the outside of the soil mass. The water here is soon used up and a decrease in transpiration occurs.

After it had been determined that the transpiration rate in diseased plants was greater than in the healthy an explanation was sought in the distribution and number of stomata on the leaves of the different types of plants. The raspberry lamina is of a delicate structure, its surface is of a convoluted or wavy nature and its only support is the prominent veins. When leaves are ashed considerable amounts of silicon are found. There was great difficulty therefore in stripping off suitable pieces of epidermis for the purpose of fixing in absolute alcohol so that stomatal studies could be made later. The collodion film method first suggested by Neuwirth (29) and later improved by Long and Clements (26) was not successful. The chief thing that militated against any method was the fact that the surface of the leaf was covered by a mat of long, tenuous interlocking hairs. It was difficult also to see the stomata because of the dense cell contents. The stomata are small and therefore quite numerous and some method had to be devised to avoid these difficulties.

First it seemed necessary to use some clearing agent. A lactic acid-phenol preparation as reported by Davis (11) proved very good. The method of counting and studying stomata consisted first in selecting suitable comparable leaves, detaching them from the parent plant and immersing them in absolute alcohol to fix them in the form in which they were gathered. The leaves were next immersed in the clearing fluid in which they remained for 24 hours and after clearing were washed with water so as to be free of clearing fluid. The tissue was stained with an aqueous solution of iodine and potassium iodide. This reagent turned the starch in the guard cells a dark blue color and it was not difficult then to find the stomata and count them. Slides were made and studied under high magnification of the microscope. Stomata were found only on the lower face of the leaf, and on the margin of the leaf where they occur in larger numbers than on the blade of the leaf. Stomatal counts are found in Table 8. Counts were taken at random in different regions of the blade, some near the mid-rib, others near the tip of the leaf, some at the base and others on the margin. Counts on 10 different microscopic fields were usually obtained.

TABLE 10. *Number of Stomata per sq. mm. of Leaf Area in Healthy and Mosaic-infected Plants.*

	Healthy						Mosaic				
	Random Counts				Margin		Random Counts			Margin	
	Old	Old	Old	Young	Young	Old	Old	Old	Young	Young	Old
1	14	14	11	21	21	16	20	25	51	45	30
2	5	10	12	13	15	14	23	30	53	30	33
3	5	11	14	14	13	21	34	22	27	52	29
4	5	9	8	15	14	14	32	26	32	46	36
5	11	13	7	13	16	15	26	34	39	38	31
6	8	8	5	21	11	14	30	28	37	29	34
7	11	10	14	11	16	16	32	31	44	49	27
8	8	9	11	16	18	12	25	24	50	52	30
9	13	12	9	16	15	13	27	23	49	48	31
10	14	11	7	11	16	15	29	30	35	36	35
Average	9.4	9.7	9.8	15.5	15.0	15.0	26.8	25.5	41.7	40.5	31.6

A comparison was made of old and young leaves. The number of stomata is different for the two types indicated. The younger leaves regardless of the fact that they may be from healthy or mosaic-infected plants, will possess, per unit area a greater number of stomata than older leaves. Also diseased leaves will have twice as many as healthy ones. This increased number may be accounted for, perhaps, by the fact that the diseased leaf is smaller than the healthy one and therefore more stomata will be collected in a unit area than on a well expanded healthy leaf. The question, however, is not one of finding an increased number of stomata but one of determining whether all or only a few are functioning. It was impossible to determine the size of the opening between the guard cells because of the unevenness of the leaf surface and the presence of an abundance of intertwining hairs. Harrison (18) reported in the case of bean mosaic that there was a reduction in transpiration and accounted for it by demonstrating a decrease in the number of functioning stomata. In fact he found many stomata closed, although the number did not vary in the two sorts of plants.

LEAF ASH STUDIES

Rao and Sreenivasaya (29) made a study of the ash of healthy and "spiked" sandal and found less calcium and potassium in "spiked" tissue than in healthy, but the writer has been unable to find any further literature on ash analysis in healthy and mosaic-infected tissues. Only one set of tests was made with raspberry plants and further work should be done to clear up discrepancies and establish tentative conclusions. Leaves of healthy, masked, and mosaic-infected red raspberry plants were collected separately and ashed at a temperature of approximately 550° C.

The elements (Ca, Fe, Mg, P, and K) in the ash were determined by the spectrographic method and the tests were made under the direction

TABLE 11. *Dry Weight, Weight of Ash, Percentage of Ash and Minerals Present in Ash of Leaves from Healthy, Masked and Mosaic-infected Plants.*

Plant	Dry Weight	Weight Ash	Per cent Ash	*Element Per cent of Ash				
				Ca.	Mg.	P	Fe	K
	Gm.	Gm.						
Healthy—A. M.....	4.834	0.463	9.57	10.7%	4.5%	3.7%	3.4%	16.0%
Healthy—P. M.....	3.383	.323	8.40	15.6	6.1	3.6	2.4	15.3
Masked—A. M.....	2.716	.269	9.90	6.3	4.7	3.0	3.4	19.0
Masked—P. M.....	3.117	.284	9.11	2.9	4.3	3.1	2.4	18.0
Mosaic —A. M.....	3.111	.290	9.32	10.5	5.0	5.3	3.7	17.3
Mosaic —P. M.....	2.687	.232	8.63	7.2	3.2	3.5	2.4	17.3

*Large quantities of silicon were found in all plants.

of Prof. D. T. Ewing of the Michigan State College Chemistry Department. The data may be found in Table 11. The percentage of ash is calculated on the dry weight basis. It may be observed from a study of the table that the afternoon figures are lower than the morning figures indicating that possibly a diurnal variation in salt content exists.

Potassium is more abundant than any other element and iron is the least plentiful. Calcium follows potassium as the next most abundant. When diseased and healthy plants are compared it is obvious that there is less calcium and more potassium in mosaic-infected leaves. The difference is not great and may not be significant but if potassium or calcium play any important rôle in photosynthesis as some think, these studies do not bear out such conclusions. One would expect to find greater quantities of these elements in plants where photosynthesis occurs most rapidly or in this case in the healthy plants. Since magnesium is an integral part of the chlorophyll molecule, and since the chlorophyll content of diseased plants is appreciably higher, one might expect a higher content of magnesium, but there is hardly a difference between the healthy and diseased plants in this respect. Wolf's (43) numerous tables do not include data on the raspberry and the writer has not been successful in finding in the literature any figures on the elements present in the raspberry plant. An abundance of silicon was found. In fact, it interfered, at first, in the preparation of samples for spectographic analysis.

DISCUSSION

It has been known for many years that when a raspberry planting becomes infected with mosaic the plants gradually decline in productivity and the planting is usually described as having "run out". The data presented in this paper may be interpreted in the main as offering an explanation for "running out" of plantings of susceptible varieties.

It is shown that production of carbohydrates is reduced by the presence of the virus and that its influence upon photosynthesis is evident. Naturally this leads to a decrease in the supply of reserves stored in the roots to be used in the succeeding crop of fruit and new canes. Progressive dwarfing of the canes produced each year in an infected planting bears out these observations. The formation of fruit of poor quality and flavor is also attributable to a reduction in photosynthesis.

In addition to a reduction in carbohydrate manufacture, it has also been possible to show that respiration is greatly increased. A high respiratory rate indicates that the products of photosynthesis are being used up much more rapidly in the mosaic-infected plants. The virus, perhaps, is wasteful of the stored energy of the diseased plant. This rapid release of energy together with the decrease in rate of formation of respirable material soon leads to starvation and eventually death. The scarcity of starch in diseased leaves is one incident, at least, of starvation conditions. Starch may be considered as one of the reserve forms of carbohydrate and in this case the simple materials are not converted into starch. If starch is formed it is soon hydrolyzed to produce respirable simple sugars when synthesis is slowed up or inactive. That something of this sort has taken place is indicated by the poor fruiting habits of the mosaic-infected plants.

Results of nitrogen analyses show that plants in which the virus is present do not contain so great a quantity of total nitrogen as healthy plants. In the ammonia fraction, however, the reverse is true. It is generally agreed that ammonia is normally used by plants in the formation of proteins therefore an increase in ammonia in the diseased plants indicates that the virus has interfered with the processes of protein synthesis.

Carbohydrate studies in this paper show lower percentage of carbohydrates in the diseased plants. This in connection with the accumulation of ammonia, indicates that the plants are unable to form proteins from the ammonia because of a lack of carbohydrates. Eventual starvation of the plant is the result of this failure of the synthetic processes and that starvation does occur is shown by the dwarfed growth made by infected shoots.

The data also show that the roots of diseased plants contain smaller quantities of all forms of storage material. This condition is probably due to the inability of these plants to synthesize materials and to the loss of material through excessive respiration of the plants in which the virus is present.

SUMMARY

This paper reports the results of investigations dealing with the physiological activities of healthy, masked, and mosaic-infected raspberries. The studies were confined to the Latham variety infected with the red raspberry mosaic.

Analyses of the leaves for various carbohydrate fractions were made. A decrease in simple sugars and starches occurred in the mosaic and masked plants. Sucrose occurred in increased amounts in mosaic-infected plants.

Total nitrogen occurred in smaller quantities in mosaic-infected plants. Nitrate nitrogen was found to be lower, while ammonia occurred in greater amounts in those plants.

Diastatic activity was studied and appeared to be accelerated in plants exhibiting the mosaic symptoms.

Photosynthetic activity and respiratory rate were determined by the CO_2 absorption method. Photosynthesis was decreased in plants showing marked symptoms of disease.

Respiration proceeds at a higher rate in diseased tissue regardless of whether the symptoms are masked or evident.

In experiments where standardized hygrometric paper was used to determine the quantity of transpiration, it was found that a higher rate of water loss was exhibited by the leaves of mosaic-infected plants.

An increase in the number of stomata per unit area of leaf tissue probably accounts for the higher rate of transpiration in the diseased leaf.

Chlorophyll determinations show a greater amount of chlorophyll per unit area of leaf in the mosaic-infected plants.

Ash analyses of the leaves of the different plants were made by the spectrographic method. This one test indicated a high amount of silicon, a greater percentage of ash in the morning material of all plants than that in the material gathered in the evening. A lower calcium content and a slightly higher percentage of potassium was found in the diseased tissues.

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